

## Research Article

# Effect of Auxin (NAA, IBA and IAA) in Root Regeneration through In vitro Culture of Sugarcane

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**Submitted:** 28 November 2018

**Accepted:** 17 December 2018

**Published:** 31 December 2018

**ISSN:** 2333-6668

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**OPEN ACCESS****Keywords**

- Sugarcane
- In vitro culture
- Auxin (NAA, IBA and IAA)
- Root regeneration

**Abstract**

An experiment containing shoot regeneration has been conducted at the Laboratory of Breeding Division of Bangladesh Sugarcane Research Institute (BSRI) Ishurdi, Pabna during the period of 2011. The present study was conducted to standardize a suitable protocol of *in vitro* plant regeneration potentiality of five sugarcane varieties. After shoot proliferation, the mini regenerated shoots were separated aseptically and cultured in MS medium containing five concentrations (viz. 1.0, 2.0, 3.0, 4.0 and 5.0mg/l) of NAA for adventitious root induction. The concentration of 5mg/l of NAA produced the minimum (9) days to root initiation the highest number (11.01) of root per culture and the highest number (0.21cm) of root length. All the variety showed the best root forming performance in concentration of 5mg/l of NAA.

**INTRODUCTION**

Sugarcane (*Saccharum officinarum* L.) is one of the most important cash crops of the world. It is one of the economically important foods-cum cash crops widely cultivated in the tropics to sub tropics and annually provides around 60 to 70 % of the world sugar [1].

The use of *in vitro* technology for improvement of sugarcane is an alternative to the conventional methods, because it is rapid, reliable and sustainable option [2]. Jahangir et al. [3], reported that it also allows the rejuvenation of plant material. Plant tissue culture techniques like callogenesis and somatic embryogenesis can be used for successful sugarcane propagation by controlling a lot of problems which are faced during conventional breeding practices. Plant regeneration from somatic embryos is a viable alternative for large scale production of plants and offers a possibility for sugarcane crop improvement [4-6]. It can be employed as an efficient way of propagation with the goal of enhancing the rate of multiplication of desired genotypes and commercial micro propagation [7]. According to Schenk and Hildebrandt [8], sugarcane requires a high concentration of auxin for rooting. Roots can be regenerated from *in vitro* regenerated shoots on half MS medium supplemented with NAA, IBA and IAA

[2,9]. Rooting was highly influenced by the different types and concentrations of auxing used. Khan et al. [10], and Singh [11], reported on the IBA and IAA, NAA was the most efficient auxing for root initiation of sugarcane *in vitro* propagation. Jagadeesh et al. [12], reported NAA was better than IBA either alone or in combination with other hormones for rooting of sugarcane. In general, many researchers such as Sandhu et al. [13], Karim et al. [14], Yadav et al., [15] and Pathak et al. [16], reported that 5mg/l NAA was the best for rooting, but more than 5mg/l NAA inhibits rooting Biradar et al., [4]. *In vitro* regeneration of sugarcane has also been reported by Heinz et al. [17]. *In vitro* selection of favorable soma clonal variation from callus culture is a supplementary tool to traditional breeding for production of stress resistant plant [18-20].

The present study is the regeneration potential of sugarcane varieties with supplementation of auxins in sugarcane on defined MS media for root induction *in vitro* culture of sugarcane. The investigation was undertaken to determine the optimum doses of auxin, the rooting medium for inducing healthy roots and makes micro propagation method economically viable and technically feasible to establish the *in vitro* regeneration and rapid propagation technique of field grown sugarcane.

## MATERIALS AND METHODS

The experiment was conducted at the Laboratory for the effect of auxin on root regeneration *in vitro* culture of sugarcane. Regenerated micro-shoots were cultured on media supplemented with different concentrations of NAA, IBA and IAA (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) individually for root formation. The sub-cultured roots continued to proliferate and in some of the cultures they differentiated into roots. The shoots grew about 3-5 cm in length with 2-3 well developed leaves were rescued aseptically from the test tubes and were separated from each other and again cultured on freshly prepared medium containing optimum concentration of NAA for rooting. All these operations were carried out under aseptic conditions and cultures were incubated at  $28 \pm 2^\circ\text{C}$  with a 16 hours photoperiod. To maintain and ensure aseptic condition precautions were taken in every step of works. All inoculation and aseptic manipulation were carried out by using a laminar air flow cabinet. Hands were properly washed with soap before starting work in laminar airflow cabinet. During the operation hands were rubbed with 70% ethyl alcohol frequency with cotton and wiped cabinet base for maintaining clean condition. Data was analyzed by using Duncan Multiple range test. Data were recorded on days to root initiation, number of roots per culture and root length (cm).

## RESULTS AND DISCUSSION

### Varietal response to application of NAA on root production

The success of *in vitro* propagation relies on efficient rooting in regenerated shoot and their subsequent acclimatization. Once the sufficient number of shoots have been generated, portion of explants that contains one or more shoots could be transferred to a medium that contains higher concentration of auxin, resulting in root formation.

Varietal response to media supplemented with auxin (NAA) on MS medium for root initiation from *in vitro* micro-shoot culture of sugarcane is shown in Table 1,2.

The highest days to root initiation (14.60) were found in Isd 37. The early days to root initiation (11.80) was showed in Isd 2-54 which were preferable for shoot induction. Among the varieties, the highest number of roots per culture (8.62) and the highest root length (0.52cm) were found in LJ-C and Isd 2-54 respectively. The lowest number (4.33) of roots per culture was observed in Isd 37 and the lowest (0.25cm) root length found in Isd 17 respectively. Gopitha et al. [21], reported that the different varieties respond differently for producing roots *in vitro* culture.

### Hormonal effect of NAA on root development

Effects of different concentration of hormone (NAA) on MS medium for root initiation from *in vitro* micro-shoot culture are shown in Table 3.

The minimum days to root initiation (9) was found in concentration of 5mg/l of NAA which were preferable for root induction. The concentration of 5mg/l of NAA produced the highest (11.01) number of root per culture and the lowest (0.21cm) root length. 1mg/l of NAA showed the lowest number of root per culture and the highest number of root length (0.56cm).

**Table 1:** MS/modified MS media supplemented with different concentrations of auxin for root regeneration.

MS + Auxin (NAA)	MS + Auxin (IBA)	MS + Auxin (IAA)
NAA1.0	IBA1.0	IAA1.0
NAA2.0	IBA2.0	IAA2.0
NAA3.0	IBA3.0	IAA3.0
NAA4.0	IBA4.0	IAA4.0
NAA5.0	IBA5.0	IAA5.0

**Table 2:** Varietal response to application of auxin (NAA) on MS medium on root initiation from *in vitro* micro-shoot culture of sugarcane.

Varieties	Days to root initiation	No. of roots / culture	Root length (cm)
Isd 2-54	11.80	6.28	0.52
LJ-C	12.20	8.62	0.26
Isd 17	14.20	5.58	0.25
Isd 37	14.60	4.33	0.45
Isd 40	12.80	7.56	0.31
LSD (5%)	0.586	0.330	0.046

**Table 3:** Effects of different concentration of hormone (NAA) on MS medium for root initiation from *in vitro* micro-shoot culture of sugarcane.

Doses of NAA (mg/l)	Days to root initiation	No. of roots / culture	Root length (cm)
1.0	16.00	2.60	0.56
2.0	16.00	4.01	0.39
3.0	13.60	6.84	0.35
4.0	11.00	7.92	0.29
5.0	9.00	11.01	0.21
LSD (5%)	0.586	0.330	0.046

The doses of 5mg/l of NAA were used by many workers for root production in sugarcane tissue culture derived plants [22-25]. The present findings were agreed with the report of Karim et al. [23], who found that higher doses of NAA (5mg/l) were required for efficient root development in sugarcane. Lal1. [26], also reported that NAA was the most suitable hormone for rooting in sugarcane. Khan et al. [11], and Singh [11], reported on the IBA and IAA, NAA was the most efficient auxin for root initiation of sugarcane *in vitro* propagation. Jagadeesh et al. [12], reported NAA was better than IBA either alone or in combination with other hormones for rooting of sugarcane.

### Interaction effect of varieties and doses of NAA for root production

Results of interaction effects of variety and doses of NAA for regeneration of root from *in vitro* micro shoots of sugarcane are shown in Table 4. Significant variation among the interaction effect of varieties and NAA for days to root initiation which ranged from 7.00 to 17.00 (Table 4). The early days (7) to root initiation was found in Isd 2-54 in concentration of 5mg/l of NAA followed by 8 days in LJ-C and Isd 40, 11days in Isd 17 and Isd 37. These findings were similar with the results of Baksha et al.

[9], who used 5mg/l NAA for the best rooting response in half strength MS medium.

The highest number (12.46) of roots per shoot was found in Isd 2-54 with rooting media 5mg/l NAA followed by 12 in Isd 40, 11.40 in LJ-C, 9.20 in Isd 37 and 7.60 in Isd 17. The lowest number (1.80) of root per culture was found in Isd 37 in concentration of 1mg/l of NAA. The dose of 5mg/l of NAA was found to perform better in root induction for all the varieties. This result agreed with result of Chegalrayan et al. [27], and Geetha et al. [28], who observed that all varieties showed better response on MS medium supplemented with 5mg/l of NAA. The highest root per shoot was also obtained in the variety Isd 28 using NAA 5mg/l by Baksha et al., [9]. The highest (0.80cm) root length was found in Isd 2-54 in the concentration of 1mg/l NAA followed by (0.76cm) in Isd 37. The lowest root length (0.16cm) was found in Isd 17 on rooting media of 5mg/l of NAA. The concentration of 5mg/l NAA produced the lowest days to root initiation and the highest number of roots per culture in Isd 2-54. The lowest root length was found in Isd 17. The present findings agreed with the report of Gopitha et al. [21], Karim et al. [23], Baksha et al. [9], Chegalrayan et al. [27], and Geetha et al. [28], and Lal [26] observed that all varieties showed better response on rooting media of 5mg/l of NAA.

### Varietal response to application of IBA for root development

Varietal response to media supplemented with IBA (auxin) on root initiation from *in vitro* micro shoots culture of sugarcane are shown in Table 5.

The lowest number of roots per culture (3.81) was found in Isd 37. The lowest root length (0.21cm) was found in variety Isd 17. Among the varieties, the early days to root initiation (12.00) was observed in LJ-C. The highest number of shoots per culture (5.04) and the highest root length (0.41cm) were found in Isd 40 and Isd 2-54 respectively. Gopitha et al. [21], reported that different varieties were found to response differently in producing roots. Similar results were obtained in this study.

### Hormonal effect of IBA for root development

Results of effects of different concentrations of IBA for root initiation from *in vitro* micro-shoot culture of sugarcane are shown in Table 6.

The concentration of 5 mg/l IBA was the best for early root initiation (10.13) which is suitable for root induction, the highest number (7.28) of roots per culture and the lowest root (0.23cm) length. The concentration of 1mg/l of IBA showed the lowest number of root per culture (2.20) and the highest root length (0.41cm). Gopitha et al. [21], reported response of different concentrations of IBA to production of roots in sugarcane. Similar results were obtained in this study.

### Interaction effect of varieties and doses of IBA on root development

Interaction effects of variety and IBA on regeneration of root from *in vitro* micro shoots of sugarcane are shown in Table 7. The higher concentration of 5mg/l IBA was found better for root regeneration in all the variety. The variety Isd 40 and LJ-C were found to require the lowest (9) days to root initiation, the highest number (8.53) of roots per culture (8.53) was observed

**Table 4:** Interaction effects of variety and NAA for regeneration of root from *in vitro* micro shoots of sugarcane.

Variety and doses of NAA (mg/l)	Days to root initiation	No. of roots / culture	Root length (cm)
Isd 2-54 × 1.0	15.00	2.33	0.80
× 2.0	15.00	3.00	0.63
× 3.0	12.00	6.00	0.50
× 4.0	10.00	7.60	0.40
× 5.0	7.00	12.46	0.30
LJ-C × 1.0	15.00	3.60	0.40
× 2.0	15.00	7.00	0.36
× 3.0	13.00	10.66	0.28
× 4.0	10.00	10.46	0.28
× 5.0	8.00	11.40	0.21
Isd 17 × 1.0	17.00	2.26	0.42
× 2.0	17.00	2.46	0.20
× 3.0	14.00	5.60	0.25
× 4.0	12.00	7.60	0.23
× 5.0	11.00	10.00	0.16
Isd 37 × 1.0	17.00	1.80	0.76
× 2.0	17.00	2.60	0.60
× 3.0	15.00	4.13	0.43
× 4.0	13.00	3.93	0.28
× 5.0	11.00	9.20	0.20
Isd 40 × 1.0	16.00	3.00	0.45
× 2.0	16.00	5.00	0.35
× 3.0	14.00	7.83	0.30
× 4.0	10.00	10.00	0.25
× 5.0	8.00	12.00	0.20
LSD (5%)	1.312	0.738	0.103

**Table 5:** Varietal response to application of IBA (auxin) on root initiation from *in vitro* micro shoots culture of sugarcane.

Varieties	Days to root initiation	No. of roots / culture	Root length (cm)
Isd 2-54	13.00	4.70	0.41
LJ-C	12.00	4.49	0.29
Isd 17	13.93	4.08	0.21
Isd 37	14.60	3.81	0.41
Isd 40	12.20	5.04	0.27
LSD (5%)	0.592	0.052	0.032

in Isd 2-54 and the lowest root length (0.12cm) was found in Isd 17. One mg/l of IBA produced the highest days to root initiation and the highest root length (0.55cm) in Isd 37. Gopitha et al. [21], reported different response of varieties with different concentrations of IBA in root development of micro-shoot of sugarcane. Similar results were obtained in this experiment. In all the varieties were found to produce lower roots in lower concentration of IBA.

### Varietal response to application of IAA on root development

Varietal response to media supplemented with IAA (auxin) for root initiation from *in vitro* micro -shoots culture of sugarcane

**Table 6:** Effects of different concentration of IBA for root initiation from *in vitro* micro-shoot culture of sugarcane.

Doses of IBA (mg/l)	Days to root initiation	No. of roots / culture	Root length (cm)
1.0	15.20	2.20	0.41
2.0	15.00	2.50	0.35
3.0	13.80	4.01	0.31
4.0	11.60	6.13	0.27
5.0	10.13	7.28	0.23
LSD (5%)	0.592	0.052	0.032

**Table 7:** Interaction effects of variety and hormone (IBA) on regeneration of root from *in vitro* micro shoots of sugarcane.

Variety and doses of IBA (mg/l)	Days to root initiation	No. of roots /culture	Root length (cm)
Isd 2-54 × 1.0	15.00	2.20	0.50
× 2.0	15.00	2.33	0.43
× 3.0	14.00	4.20	0.41
× 4.0	11.00	6.26	0.37
× 5.0	10.00	8.53	0.35
LJ-C × 1.0	14.00	2.20	0.46
× 2.0	14.00	2.80	0.30
× 3.0	13.00	4.26	0.25
× 4.0	10.00	6.40	0.23
× 5.0	9.00	6.80	0.21
Isd 17 × 1.0	16.0	2.00	0.27
× 2.0	16.00	2.20	0.23
× 3.0	14.00	3.60	0.22
× 4.0	13.00	6.00	0.19
× 5.0	10.67	6.60	0.12
Isd 37 × 1.0	17.00	1.80	0.55
× 2.0	16.00	2.00	0.48
× 3.0	15.00	3.00	0.40
× 4.0	13.00	5.80	0.33
× 5.0	12.00	6.46	0.30
Isd 40 × 1.0	14.00	2.80	0.35
× 2.0	14.00	3.20	0.30
× 3.0	13.00	5.00	0.30
× 4.0	11.00	6.20	0.25
× 5.0	9.00	8.00	0.17
LSD (5%)	1.325	0.332	0.073

are shown in Table 8.

Among the varieties, the shortest day to shoot initiation (13.00) was found in variety Isd 40. The highest number of root per culture (3.88) and the lowest root length (0.25cm) were found in LJ-C. The highest root length (0.38cm) and the lowest number of root per culture (2.36) were observed in Isd 2-54 and Isd 37. Gopitha et al. [21], reported varietal response under different concentrations of IAA for root development in sugarcane. Similar results were obtained in this study.

## Hormonal effect of IAA on root development

Effects of different concentrations of IAA for root initiation from *in vitro* shoot culture of sugarcane are shown in Table 9. The concentration of 5mg/l of IAA produced the highest number of roots per culture (4.68) and the lowest root length (0.19cm). One mg/l of IAA showed the highest days to root initiation (16.40), highest root length (0.47cm) and the lowest number of root per culture (1.81). Gopitha et al. [21], reported differential response of concentrations of IAA in root production of sugarcane. Similar results were obtained in this study.

## Interaction effect of varieties and doses of IAA on root development

Interaction effects of variety and hormone (IAA) on regeneration of root from *in vitro* micro shoots of sugarcane are shown in Table 10. The concentration of 4mg/l of IAA showed the early days to root initiation (11) in Isd 40. The doses of 5mg/l of IAA produced the highest number of root per culture (6.33) and the lowest root length (0.15cm). The highest root length (0.60cm) was found in 1mg /l of IAA. Gopitha et al. [21], reported that the different concentrations of IAA were found to be better responsive for producing roots. Similar results were obtained in this experiment.

## CONCLUSIONS

The doses of 5mg/l of NAA are suitable for growth and development of roots in shoots. The interaction effect of varieties and NAA for root initiation which ranged from 7 to 17. The early days (11.8) to root initiation was found in Isd 2-54 in concentration of 5mg/l of NAA. The concentration of 5mg/l of NAA showed the best root formation of all varieties.

**Table 8:** Varietal response to application of IAA (auxin) for root development *in vitro* micro -shoots culture of sugarcane.

Varieties	Days to root initiation	No. of roots / culture	Root length (cm)
Isd 2-54	14.00	3.05	0.38
LJ-C	13.73	3.88	0.25
Isd 17	15.20	2.58	0.33
Isd 37	14.00	2.36	0.37
Isd 40	13.00	3.28	0.30
LSD (5%)	0.610	0.167	0.040

**Table 9:** Effects of different concentrations of IAA for root initiation from *in vitro* shoot culture of sugarcane.

Doses of IAA (mg/l)	Days to root initiation	No. of roots / culture	Root length (cm)
1.0	16.40	1.81	0.47
2.0	15.93	2.36	0.41
3.0	14.00	2.82	0.29
4.0	11.20	3.48	0.28
5.0	12.40	4.68	0.19
LSD (5%)	0.610	0.148	0.040

**Table 10:** Interaction effects of variety and IAA on regeneration of root from *in vitro* micro shoots of sugarcane.

Variety and doses of IAA (mg/l)	Days to root initiation	No. of roots / culture	Root length (cm)
Isd 2-54 × 1.0	17.00	2.00	0.60
× 2.0	15.00	2.20	0.40
× 3.0	13.00	3.06	0.33
× 4.0	12.00	3.20	0.40
× 5.0	13.00	4.80	0.20
LJ-C × 1.0	16.00	2.00	0.40
× 2.0	15.00	3.26	0.30
× 3.0	14.00	3.60	0.21
× 4.0	11.00	4.20	0.21
× 5.0	12.00	6.33	0.15
Isd 17 × 1.0	18.00	1.60	0.40
× 2.0	18.00	2.06	0.55
× 3.0	15.00	2.46	0.28
× 4.0	12.00	3.00	0.23
× 5.0	13.00	3.80	0.21
Isd 37 × 1.0	16.00	1.60	0.56
× 2.0	16.00	1.80	0.50
× 3.0	14.00	2.00	0.30
× 4.0	11.00	2.80	0.27
× 5.0	13.00	3.60	0.23
Isd 40 × 1.0	15.00	1.86	0.40
× 2.0	15.00	2.46	0.31
× 3.0	14.00	3.00	0.33
× 4.0	10.0	4.20	0.30
× 5.0	11.00	3.86	0.19
LSD (5%)	1.365	0.374	0.089

## ACKNOWLEDGEMENT

The authors are thanks to the scientist of breeding division of Bangladesh Sugar crop Research Institute for their technical support. I also wish to gratefully acknowledge the financial assistance from the ministry of Science and Information & Communication Technology of Bangladesh for supporting NSICT fellowship to carry the research.

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#### Cite this article

Rahman MM, Ivy NA, Mian MA, Rasul MG, Hossain MM, et al. (2018) Effect of Auxin (NAA, IBA and IAA) in Root Regeneration through In vitro Culture of Sugarcane. *Int J Plant Biol Res* 6(6): 1109.